

# *Environmental Effects of Dredging Technical Notes*



## A PLANT BIOASSAY FOR ASSESSING PLANT UPTAKE OF HEAVY METALS FROM CONTAMINATED FRESHWATER DREDGED MATERIAL

**PURPOSE:** The Decisionmaking Framework (DMF) developed by Peddicord et al. (1986) provides a framework for evaluating sediments before dredging. This framework is comprised of several modules one of which is the Plant Bioassay for materials proposed for upland or wetland placement. The purpose of this note is to describe the methods and materials necessary to conduct such a plant bioassay.

**BACKGROUND:** The US Army Engineer Waterways Experiment Station (WES) has developed a plant bioassay using the freshwater plant *Cyperus esculentus* to evaluate phytotoxicity and potential mobility of contaminants from dredged material into the environment through plant uptake (Folsom and Lee 1981; Folsom, Lee, and Bates 1981). The plant bioassay procedure is an excellent tool for predicting bioaccumulation of heavy metals (e.g. zinc and cadmium) from freshwater sediments (Lee, Folsom, and Bates 1983). The bioassay was successfully evaluated using metal-contaminated Dutch sediments (Van Driel, Smilde, and van Luit 1983) as well as Welsh mining wastes (Folsom, Davis, and Houghton, 1988). Like the DMF, the Plant Bioassay Module is based on tiered testing. Tier I is a chemical extraction of test and reference sediments; Tier II is a laboratory/greenhouse plant bioassay. If the results of Tier I testing indicate a reason for concern, then the test sediment could be subjected to Tier II testing to verify the concern. Tier II testing consists of a laboratory/greenhouse plant bioassay using test and reference sediments.

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## Sediment Collection and Preparation

Sediments to be tested are collected from a waterway using an appropriate sampler that can sample the entire vertical profile of the material to be dredged. The plant bioassay actually requires 16 gal of each sediment to be tested. Therefore, a 76- or 114-l (20- or 30-gal) drum would provide sufficient quantity of material to conduct the required testing; however, 208-l (55-gal) steel drums are generally easier to obtain. The drums are sealed with airtight lids and transported to the laboratory. Temperature during shipping should be maintained at  $4^{\circ} \pm 2^{\circ} \text{C}$ . Unless new, the drums should be steam-cleaned prior to use.

Before testing, the original, flooded sediments should be mixed thoroughly while in their respective drums using a mixer. All debris, such as cans, bottles, leaves, or twigs, is removed. The mixer is raised and lowered to thoroughly mix the sediment contained in each drum. Generally, one hour of mixing is required for adequate sediment homogenization. Testing using four replicates results in sample variability (coefficient of variation) of less than 10 percent. Subsamples from the drums represent original, flooded sediment and are used in the procedure described below.

### Flooded condition

A schematic diagram of the standard WES plant bioassay apparatus is shown in Figure 1. The mixed original, flooded sediment is placed one 500-ml scoop at a time to further minimize mixing variability, into each of four 7.6-l (2-gal) Bain-Marie containers. When the containers are filled with sediment, sediment diameter and sediment depth should be in a ratio of 1:1.5. Flooded conditions are maintained by keeping a 5-cm depth of deionized water or distilled water above the sediment surface of the inner Bain-Marie container. Percent moisture on an oven-dry weight basis (ODW) (oven temperature is  $105^{\circ} \pm 2^{\circ} \text{C}$  for 17 hr) is determined on small (5- to 10-g) subsamples of the flooded material in each replicate. A test sediment weight of 4,500 g (ODW) per replicate generally provides sufficient plant material for maximum plant growth. Since flooded and upland biomass production (yield) is one of the Decisionmaking Framework (DMF) comparisons, each replicate must contain the same quantity of sediment (ODW). The four flooded replicates are sealed with their lids and stored at  $4^{\circ} \pm 2^{\circ} \text{C}$  until the upland replicates (described below) are prepared for planting. Drying

and preparation of the upland replicates should be completed within three weeks of collection.

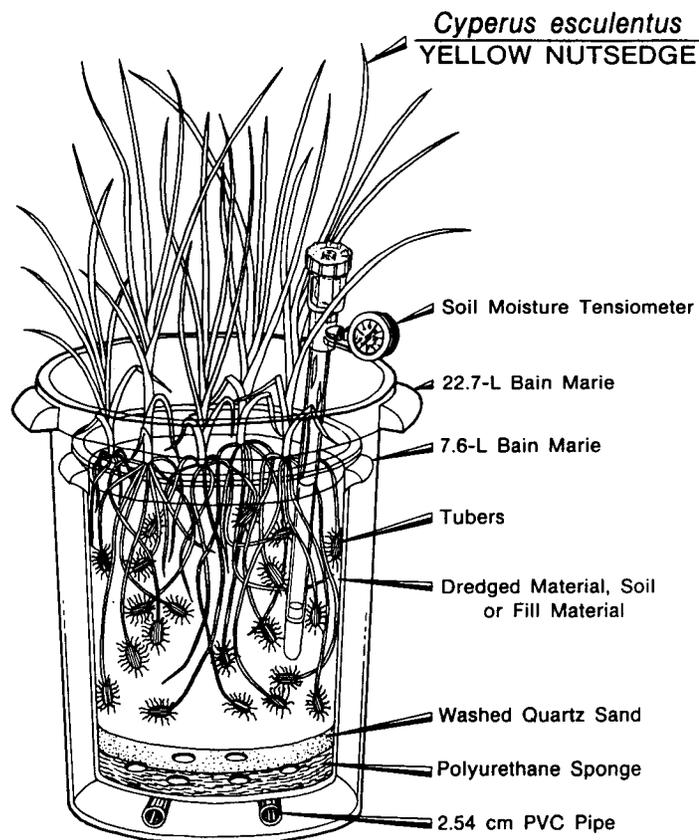


Figure 1. Schematic diagram of the Plant Bioassay Apparatus; sand layer and sponge are each 2.54 cm thick

### Upland condition

Four additional sediment replicates are prepared as described above for the flooded condition except that the sediment from each container is placed into an aluminum drying pan and allowed to air dry. The sediment must be turned and mixed daily with a large plastic spatula or Teflon-coated shovel to facilitate drying. All debris, such as cans, bottles, leaves, or twigs, is removed as the sediment dries. If large quantities of large rocks, gravel, and other materials are present, then a separation analysis should be conducted (Engineer Manual 1110-2-1906, Appendix V) (Headquarters, US Army Corps of Engineers 1970). After air-drying the sediment, most sediments form large bricklike clods that are extremely difficult to crush. Crushing and grinding of these clods is best

performed using a hammermill. Personnel operating the hammermill should wear appropriate respirators and protective clothing. One pass through the hammermill is sufficient for the material to pass a 2-mm screen (U.S. Standard Sieve No. 10). Greenhouse pot experiments generally use material that has been ground to pass a 2-mm screen to approximate field macroporosity (pore space affects particle surface area, drainage, gas movement, and other items) to estimate weathered sediment placed in an upland disposal site. The screened material is returned to a drying flat where it is remixed and subsampled for ODW analysis. Air-dried sediment (4,500 g ODW) is placed (one 500-ml scoopful at a time) into each of four 7.6-ℓ Bain-Marie containers prepared as before. The remaining air-dried sediment can be placed into an appropriate container (7.6-ℓ Bain-Marie bucket is a good choice), and stored until needed for subsequent chemical or physical analyses, if necessary. For air-dried replicates soil moisture is maintained between 0.04 and 0.06 MPa (field capacity is 0.00 MPa) by checking soil moisture tensiometers in each container daily. Plants are watered when tensiometers read greater than 0.06 MPa (generally every other day). When watering is necessary, the outer container is filled up to the sediment level of the inner container with distilled water. When tensiometers read less than 0.04 MPa, the water is siphoned out of the outer container.

Material from the reference or proposed disposal site is prepared in exactly the same manner as that described above for each disposal condition (i.e. flooded and upland).

#### Greenhouse Operation

The replicates are randomly placed on tables in a greenhouse. Day length of 16 hr is maintained by using light fixtures whose face is 130 cm from the top of the 22.7-ℓ Bain-Marie container. The 130-cm height allows potential maximum plant growth to occur without contacting the light fixture or becoming so close to the light that plant tissue is damaged from excess heat. Lights should be arranged in a pattern of alternating high-pressure sodium lamp and a high-pressure multi-vapor metal halide lamp. Alternating the lamps provides an even photosynthetic active radiation (PAR) distribution pattern. The PAR should be 1,200 microeinsteins per metre squared. The temperature of the greenhouse is maintained at  $32.2^{\circ} \pm 2^{\circ}$  C maximum during the day and  $21.1^{\circ} \pm 2^{\circ}$  C minimum at night to simulate a summer environment. Relative humidity should be maintained

as closely as possible to 100 percent, but never less than 50 percent.

### Planting and Growing Techniques

The plant used in the WES plant bioassay is *Cyperus esculentus*. Normally, *C. esculentus* (common name, yellow nutsedge) is considered a persistent major problem weed and causes yield reductions in many crops of the United States, Canada (Mulligan and Junkins 1976; Wills, Hoagland, and Paul 1980), and throughout the world (Holm et al. 1977). It is also considered a pioneer species that invades disturbed areas (e.g. dredged material disposal sites) readily (Mulligan and Junkins 1976). Although *C. esculentus* reproduces by seeds, tubers, bulbs, and rhizomes, tubers are the primary means of reproduction (Bell et al. 1962; Tumbleson and Kommedahl 1961), even though it may flower and produce seed under certain conditions (Mulligan and Junkins 1976). Therefore, *C. esculentus* was chosen as the plant bioassay index plant because of its natural tenacity and its ability to survive in both flooded and upland conditions and showed greatest potential for heavy metal uptake compared to other plant species (Lee, Sturgis, and Landin 1975). *Cyperus esculentus* also has a fairly short 45-day vegetative growth period under long days (Doty and Sweet 1970).

Each replicate of flooded and upland sediment is planted with four germinated tubers of *C. esculentus*. Suppliers of the tubers include Valley Seed Services (P.O. Box 8335, Fresno, CA 93791, phone: 209-435-2763) and Wildlife Nurseries (P.O. Box 2724, Oshkosh, WI 54903; phone 414-231-3780). Because germination of *C. esculentus* is close to 50 percent (Thomas 1969; Yip and Sweet 1978), twice as many tubers as needed for the experiment are set out for germination. Germination temperature is  $23^{\circ} \pm 2^{\circ}$  C in the light. The tubers are first rinsed in distilled water to remove substances that inhibit sprouting of buds on the tubers (Mulligan and Junkins 1976). The tubers are then placed between white paper towels and kept moist with distilled water until enough have sprouted to plant five tubers per container (usually seven to ten days). Sprouts should be approximately 3 cm long before planting. Plants are allowed to grow for 45 days from the time of planting.

### Harvesting

After 45 days, the aboveground plant material from each replicate is cut 5 cm above the sediment surface with stainless steel scissors and placed into

a labeled brown paper bag perforated by several holes to allow water vapor to escape during drying. Any flowers, stems, or seeds that may have developed are separated from the leaves, wrapped separately with white paper towels, and placed into the bag with the leaves. The bags containing the harvested plant material are dried to a constant weight in a forced-air drying oven at  $70^{\circ} \pm 2^{\circ} \text{C}$  (generally four or five days). All dried tissue is removed from the bags and weighed separately. Total plant yields are determined by weighing the oven-dried plant material.

### Digestion and Chemical Analysis of Plant Material

The dried leaves are ground in a small Wiley mill. Two grams (weighed to the nearest 0.0001 g) of the ground leaf tissue are digested using the tertiary acid digestion procedure except that 2.0 g ODW tissue are used rather than 1.0 g (Folsom and Houck in preparation). In some sediments, plant growth is not sufficient to provide 2.0 g of tissue. In these cases, whatever amount of tissue produced is digested. Chemical analysis of flowers, stems, and seeds is not conducted since their production is sporadic. Care should be taken during the initial digestion because excessive frothing of the nitric acid may occur, rendering that replicate useless. The diluted digestates are analyzed for heavy metals by atomic absorption (AA) spectroscopy or heated graphite analysis (HGA). Results of the digestion are calculated using the equation:

$$\begin{aligned} \text{Tissue metal concentration} &= \frac{\text{solution metal concentration} \times \text{dilution volume}}{\text{ODW leaf tissue digested}} \\ &= \frac{\mu\text{g metal/ml} \times 50 \text{ ml}}{\text{grams tissue}} \\ &= \frac{\mu\text{g metal}}{\text{grams tissue}} \end{aligned}$$

Plant uptake or organic compounds by *C. esculentus* has been limited to studies with 2,4,6-trinitrotoluene (TNT) (Folsom et al. 1988; Pennington 1988; and Palazzo and Leggett 1986) and polychlorinated biphenyl (PCB).\*

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\* Folsom, B. L., Jr. Unpublished laboratory results, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

literature review on potential contaminant migration pathways Brannon et al. (1989) suggested that plant uptake of organic compounds may be very important to cycling of organic compounds in confined dredged material disposal facilities and recommended further research be conducted.

### Data Comparisons

Plant heavy metal concentrations, total heavy metal plant uptake, and yield data are used to make the required DMF numerical comparisons. Plant heavy metal concentration, total heavy metal uptake, and yield are subjected to analysis of variance (ANOVA) and the Waller-Duncan K-Ratio t test to determine if test sediment mean values are different from reference sediment mean values. In cases where no plants survive in a replicate, the number of surviving replicates should be reported. Results of statistical analyses are then used to make the numerical comparisons (paragraphs B50 to B52, Peddicord 1986) in the DMF to help determine the most appropriate disposal option.

### Summary

Sediments are thoroughly mixed before testing. The plant bioassay procedure is generally conducted using four replicates of each disposal condition (i.e. flooded and upland) for each sediment or reference site considered. Flooded plant bioassay replicates are prepared and stored until sediment has been air-dried for the upland plant bioassay replicates. Replicates are placed into a controlled greenhouse environment and allowed to grow for 45 days. Aboveground plant tissue is harvested, weighed, acid digested, and analyzed for heavy metals by atomic absorption spectroscopy. Generally, heated graphite analysis is required to obtain the heavy metal concentration data. Test and reference sediment results are compared and conclusions are used in the Decisionmaking Framework.

## References

- Bell, R. S., Lachman, W. H., Rahn, E. M., and Sweet, R. D. 1962. "Life History Studies as Related to Weed Control in the Northeast; 1, Nutgrass," Northeast Regional Publication, Agricultural Experiment Station Bulletin 364, University of Rhode Island, Kingston, RI.
- Brannon, J. M., Pennington, J. C., Gunnison, D., and Myers, T. E. 1989. "Comprehensive Analysis of Migration Pathways (CAMP): Migration Pathways Controlling Internal Cycling and Net Mass Transport of Contaminants at Confined Dredged Material Disposal Facilities," Miscellaneous Paper in preparation, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Doty, C. H., and Sweet, R. D. 1970. "Growing Nutsedge (*Cyperus esculentus*) in the Greenhouse for Research Purposes," *Proceedings*, Northeast Weed Science Society, No. 24, pp 302-306.
- Folsom, B. L., Jr. and Lee, C. R. 1981. "Zinc and Cadmium Uptake by the Freshwater Marsh Plant *Cyperus esculentus* Grown in Contaminated Sediments Under Reduced (Flooded) and Oxidized (Upland) Disposal Conditions," In International Symposium on Trace Element Stress in Plant, *Journal of Plant Nutrition*, No. 3, pp 233-244.
- Folsom, B. L., Jr., Davis, B. E., and Houghton, N. J. 1988. "Heavy Metal Uptake by Agronomic Crops and *Cyperus esculentus* Grown on Oxidized and Reduced Soils Contaminated with Metal-Mining Wastes," Environmental Effects of Dredging Technical Note EEDP-02-06, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Folsom, B. L., Jr., Pennington, J. C., Teeter, C. L., Barton, M. R., and Bright, J. A. 1988. "Effects of Soil pH and Treatment Level on Persistence and Plant Uptake of 2,4,6-Trinitrotoluene," Technical Report EL-88-22, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Folsom, B. L., Jr., Lee, C. R., and Bates, D. J. 1981. "Influence of Disposal Environment on Availability and Plant Uptake of Heavy Metals in Dredged Material," Technical Report EL-81-12, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Folsom, B. L., Jr. and Houck, M. H. "Test Protocol for Predicting Plant Uptake of Heavy Metals from Contaminated Freshwater Dredged Material," Environmental Effects of Dredging Technical Note in preparation, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Headquarters, US Army Corps of Engineers. 1970. "Engineering and Design; Laboratory Soils Testing; Appendix V, Grain-Size Analysis," Engineer Manual 1110-2-1906, Washington, DC.
- Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. 1977. "The World's Worst Weeds, Distribution and Biology," University Press, Hawaii.
- Lee, C. R., Folsom, B. L., Jr., and Bates, D. J. 1983. "Prediction of Plant Uptake of Toxic Metals Using a Modified DTPA Soil Extractant," *The Science of the Total Environment*, No. 28, pp 191-202.
- Lee, C. R., Sturgis, T. C., and Landin, M. C. 1976. "A Hydroponic Study of Heavy Metal Uptake by Selected Marsh Plant Species," Technical Report D-76-6, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Mulligan, G. A., and Junkins, B. E. 1976. "The Biology of Canadian Weeds," *Canadian Journal of Plant Science*, No. 56, pp 339-350.
- Peddicord, R. K., Lee, C. R., Palermo, M. R., and Francingues, N. R., Jr. 1986. "General Decisionmaking Framework for Management of Dredged Material," Miscellaneous Paper, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Palazzo, A. J., and Leggett, D. C. 1986. "Effect and Disposition of TNT in a Terrestrial Plant," *Journal of Environmental Quality*, No. 15, pp 49-52.
- Pennington, J. C. 1988. "Soil Sorption and Plant Uptake of 2,4,6-Trinitrotoluene," Technical Report EL-88-12, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Thomas, P. E. L. 1969. "Effects of Desiccation and Temperature on Survival of *Cyperus esculentus* Tubers and *Cynodon Dactylon* Rhizomes," *Weed Research*, No. 9, pp 1-8.
- Van Driel, W., Smilde, K. W., and van Luit, B. 1983. "Comparison of the Heavy-Metal Uptake of *Cyperus esculentus* and of Agronomic Plants Grown on Contaminated Sediments," Miscellaneous Paper D-83-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Willis, G. D., Hoagland, R. E., and Paul, R. N. 1980. "Anatomy of Yellow Nutsedge (*Cyperus esculentus*)," *Weed Science*, No. 28, pp 432-437.
- Yip, C. P., and Sweet, R. D. 1978. "Biotypes of Yellow Nutsedge (*Cyperus esculentus* L.): I. Tuber Dormancy and Responses to Photoperiod and Herbicides," *Weed Sciences Society of America*, Abstract No. 128, p 59.